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TITLE: The Fanconi Anemia BRCA Pathway as a Predictor of Benefit from Bevacizumab in a Large Phase III Clinical Trial in Ovarian Cancer

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14. ABSTRACT

The Gynecologic Oncology Group (GOG) trial 218 was a randomized phase III trial in primary stage III and IV ovarian carcinoma, which found a statistically significant improvement in progression free survival (PFS) for combination chemotherapy with extended bevacizumab, an antibody to the VEGF receptor that inhibits angiogenesis. We deeply sequences 65 DNA repair genes in germline and/or tumor DNA from GOG218 and another upfront clinical trial GOG262. We found that germline or somatic mutations in the BRCA-Fanconi anemia pathway was significantly associated with improved progression-free and overall survival. Moreover, the presence of inherited mutations was not limited to high grade serous carcinomas but was found in most histological sub-type. Finally, we did not identify an interaction between the presence of a BRCA-Fanconi anemia mutation and benefit from bevacizumab in GOG218.

15. SUBJECT TERMS

Nothing listed

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INTRODUCTION:
The Gynecologic Oncology Group (GOG) trial 218 was a randomized phase III trial in primary stage III and IV ovarian carcinoma, which found a statistically significant improvement in progression free survival (PFS) for combination chemotherapy with extended bevacizumab, an antibody to the VEGF receptor that inhibits angiogenesis. However, this relatively small increase in PFS came at high financial cost and increased toxicity. Identifying biomarkers that predict increased response to bevacizumab is essential to allow more individualized and cost effective patient care. Inherited mutations in *BRCA1* and *BRCA2* (*BRCA1/2*) occur in 13-18% of all ovarian carcinomas. *BRCA1*/2 are involved in the Fanconi anemia (FA) DNA repair pathway and *BRCA2* is a FA gene. Women with *BRCA1*/2 mutations are known to have an improved overall survival compared to women with sporadic ovarian carcinoma. The FA-BRCA pathway controls DNA repair via homologous recombination (HR). Recently, our group and others have more broadly implicated the FA-BRCA pathway in the etiology of hereditary ovarian carcinoma, identifying a number of new ovarian cancer susceptibility genes including *BARD1*, *BRIP1*, *RAD51C,* and *RAD51D*. Our hypothesis was that women that are wildtype for germline mutations in the FA-BRCA pathway would demonstrate greater benefit from the addition of extended bevacizumab to standard induction chemotherapy for advanced ovarian carcinoma.

KEYWORDS:
Ovarian, angiogenesis, clinical trial, homologous recombination, mutation, BRCA1, BRCA2, PALB2, BARD1, RAD51C, RAD51D, BRIP1, outcomes, histology

ACCOMPLISHMENTS:

Major goals: Our primary objective was to deeply sequence a large number of genes in the FA-BRCA pathway in germline DNA from GOG218 and correlate the presence of deleterious mutations with progression-free survival according to treatment arm. Secondarily we assessed the contribution of novel FA-BRCA genes to hereditary ovarian carcinoma including those of various histological sub-types and correlated the presence of FA-BRCA mutations with overall survival and treatment toxicity.

What was accomplished:
Major activities**:** Completed DNA sequencing using BROCA-HR assay developed by our group of 65 DNA repair genes on tumor and/or blood DNA from 1195 cases from GOG218 and from 557 blood DNAs from GOG262.

Specific Objectives:

1. Correlate the presence of deleterious mutations in HR genes with progression-free survival according to treatment arm in GOG218
2. Evaluate the rate of damaging germline mutations in BRCA-FA genes in a large series of unselected women with ovarian cancer.
3. Compare mutation rates from #2 above with a large set of publically available population data.
4. Correlate the presence of deleterious germline mutations in ovarian cancer suscpetibiliyt genes with histological sub-type.
5. Correlate the presence of deleterious germline mutations with toxicity in GOG 218.

Germline DNA results including results of objectives 2-4 above were reported in the following manuscript:

Norquist BM, Harrell MI, Brady MF, Walsh T, Lee MK, Gulsuner S, Bernards SS, Casadei S, Yi Q, Burger RA, Chan JK, Davidson SA, Mannel RS, DiSilvestro PA, Lankes HA, Ramirez NC, King MC, Swisher EM*, Birrer MJ*. Inherited Mutations in Women With Ovarian Carcinoma. JAMA Oncol. 2015 Dec 30:1-9. PMID: 26720728
*Drs. Swisher and Birrer were co-senior authors on this manuscript

Key findings from this work included the following:

1. PALB2 and BARD1 are new ovarian cancer susceptibility genes.
2. We confirmed the importance of RAD51C, RAD51D, BRIP1 as ovarian cancer susceptibility gene.
3. Other known or purported breast cancer susceptibility genes excluding BRCA1 and BRCA2 are not clearly associated with risk of ovarian cancer.
4. All histological sub-types of ovarian cancer are associated with inherited mutations in ovarian cancer susceptibility genes suggesting that all women with invasive ovarian, peritoneal of fallopian tube cancer should have genetic testing.
5. The degree of risk associated with mutations in PALB2, RAD51C, RAD51D and BRIP1 warrant consideration of age appropriate risk-reducing salpingo-oophorectomy.

The data addressing objective 1 and 5 was recently presented as a plenary presentation at the Annual Meeting of the Society of Gynecologic Oncology (SGO). This presentation won the Presidential Award for the best scientific abstract at the 2016 meeting:

Norquist BM, Harrell MI, Brady MF, Walsh T, Lee MK, Gulsuner S, Bernards SS, Casadei, Burger RA,, Davidson[6] SA, Mannel RS, DiSilvestro PA, Lankes HA, Ramirez NC, King MC, Michael J. Birrer MJ, Swisher EM Defects in Homologous Recombination and Response to Bevacizumab in GOG 218: an NRG Oncology Study. Society of Gynecologic Oncology Annual Meeting, March, 2016, San Diego, CA

A manuscript is currently in preparation.

This aspect of the work focused on the germline and somatic mutations in GOG 218. Clinical characteristics of cases used is provided in Table 1.

Table 1 Clinical Characteristics		N (%)
Total		1195
Age	<40	40 (3.3%)
	40 – 49	176 (14.7%)
	50 – 59	385 (32.2%)
	60 – 69	374 (31.3%)
	70 – 79	205 (17.2%)
	≥80	15 (1.3%)

Race/Ethnicity*	Non-Hispanic White	1048 (87.7%)
	Hispanic	51 (4.3%)
	Non-Hispanic Black	46 (3.8%)
	Asian/Pacific Islander	31 (2.6%)
	Other or Unknown	19 (1.6%)
Disease Site	Ovary	998 (83.5%)
	Peritoneal	171 (14.3%)
	Fallopian Tube	26 (2.2%)
Stage	Stage III/Optimal	465 (38.9%)
	Stage III/Suboptimal	453 (37.9%)
	Stage IV	277 (23.2%)
Histology	High-Grade Serous	971 (81.3%)
	Low-Grade Serous	46 (3.8%)
	Carcinoma, NOS	84 (6.2%)
	Low-Grade Endometrioid	4 (0.3%)
	High-Grade Endometrioid	38 (3.2%)
	Clear Cell	28 (2.3%)
	Mucinous	7 (0.6%)
Treatment Arm	CT + P -> P	408 (34.1%)
	CT + B -> P	386 (32.3%)
	CT + B -> B	401 (33.6%)

Abbreviations: High-Grade (grades 2 and 3), Low-Grade (grade 1), NOS (not otherwise specified), CT (chemotherapy), P (placebo), B (Bevacizumab).

A summary of the samples sequenced and results is provided in Table 2.

Table 2:

Source of Sequenced DNA		DNA from Blood (Detects Germline Mutations)			
			DNA from Tumor (Detects Both Germline and Somatic Mutations)		
		*Blood (Germline Only)	**Blood and Tumor (Somatic Only)	Tumor Only (Germline and Somatic)	All Sources
N (%)		464 (38.8)	324 (27.1)	407 (34.0)	1195 (100)
Mutation Group	*BRCA1* (%)	79 (10.0)	17 (5.2)	52 (12.8)	148 (12.4)
	BRCA2 (%)	46 (5.8)	7 (2.2)	25 (6.1)	78 (6.5)
	Other HR (%)	50 (6.3)	8 (2.5)	23 (5.7)	81 (6.8)
	Total HR (%)	175 (22.2)	32 (9.9)	100 (24.4)	307 (25.7)

*Percentages with mutations calculated out of the total 788 patients with germline testing
**Subset of the 788 patients with germline testing who had previously negative germline testing, therefore these mutations are somatic only

Both progression-free survival (PFS) and overall survival (OS) was significantly better for cases with *BRCA1*, *BRCA2* or other HR mutations in comparison to wild-type (no mutations) (Figure 1). For the purposes of these analyses other HR mutations was defined as including damaging mutations in the following genes: *ATM*, *ATR*, *BARD1*, *BLM*, *BRIP1*, *CHEK2*, *MRE11A*, *NBN*, *PALB2*, *RAD51C*, *RAD51D*, *RBBP8*, *SLX4*, and *XRCC2*.

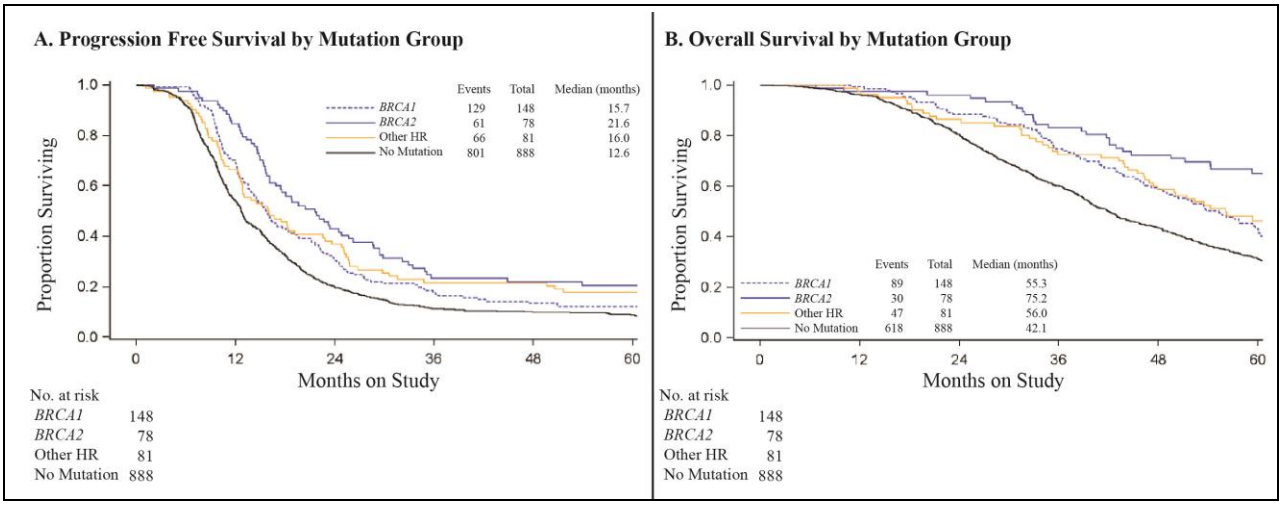

Figure 1.

Hazard ratios were significant for progression (Table 3) or death (Table 4) after adjustment for major prognostic variables including study treatment, stage of disease, size of residual disease, and initial performance status.

Table 3. Adjusted Hazards for Progression by Mutation Category

Gene Category	Total N	Events (%)	aHR (95% CI)	P-value
BRCA1	148	129 (87.2)	0.80 (0.66 – 0.97)	0.02
BRCA2	78	61 (78.2)	0.52 (0.40 – 0.67)	<0.0001
Other HR	81	66 (81.5)	0.73 (0.57 – 0.94)	0.01
No Mutation	888	801 (90.2)	1.0 (referent)	Referent

Table 4. Adjusted Hazards for Death by Mutation Category

Gene Category	Total N	Events (%)	aHR (95% CI)	P-value
BRCA1	148	89 (60.1)	0.80 (0.66 – 0.97)	0.02
BRCA2	78	30 (38.5)	0.52 (0.40 – 0.67)	<0.0001
Other HR	81	47 (58.0)	0.73 (0.57 – 0.94)	0.01
No Mutation	888	618 (69.6)	1.0 (Referent)	Referent

Events (progression or death in 60 months), aHR (adjusted hazard ratio).
The no mutation group was the referent group.

Toxicities were not significantly different by mutation group.

Using a test of interaction, there was no interaction between the effect of bevacizumab and mutation status on PFS or OS, suggesting that mutation status should not be used to select which patients should get adjuvant bevacizumab in the primary treatment of advanced ovarian cancer. This is a key

finding of the study.

Opportunities for training and professional development provided: Dr. Barbara Norquist completed much of the sequencing on this project as a new faculty clinician scientist and had the opportunity for one on one mentoring on sequencing and analyses with Drs. Swisher, Walsh and King. She also was mentored by Dr. Mark Brady on the statistical analyses.

How were the results disseminated to communities of interest?
Results were disseminated to the scientific and lay community through the manuscript and abstract listed above (and included in the Appendix). An additional manuscript is under preparation.

What do you plan to do during the next reporting period to accomplish the goals?
Nothing to report.

IMPACT
Important findings from this research are impactful for the ovarian cancer community. We determined that mutation status including the presence of BRCA1, BRCA2, and other HR mutations should not be used to select which patients get adjuvant bevacizumab in the primary treatment of advanced ovarian cancer. We found that BRCA1 and BRCA2 and other HR mutations have a strong influence on overall survival and these factors should be assessed in clinical trials of ovarian cancer. We also found that BRCA1 and BRCA2 and other HR mutations are present in all histological sub-types and clinical trials that assess PARP inhibitors and include only high grade serous cancers are missing a significant fraction of HR-deficient ovarian cancers that could potentially benefit from these therapies. We also identified PALB2 and BARD1 as new ovarian cancer susceptibility genes, which has important indications for predicting risk and preventing ovarian cancer. Overall, we determined that at least 11 genes contribute to ovarian cancer and explain 20% of cases suggesting that 20% of ovarian cancers could be predicted and therefore targeted for prevention.

What was the impact on other disciplines? Nothing to report

What was the impact on technology transfer? Nothing to report

What was the impact on society beyond science and technology? By better identifying which genes cause ovarian cancer, we have provided an opportunity to improve public education about the importance of genetic factors as a risk factor for ovarian cancer. We are actively pursuing the goal to increase public awareness through advocacy organizations including The Ovarian Cancer Research Fund Alliance (OCRFA), the National Ovarian Cancer Coalition (NOCC) and Stand up to Cancer (SU2C). Below is an example of one of our efforts attempt to raise public awareness.
https://www.yahoo.com/katiecouric/q-and-a-with-dr-elizabeth-swisher-172612116.html

CHANGES/PROBLEMS: Nothing to report

PRODUCTS: Nothing to report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS
What individuals have worked on the project?

Name:	*Elizabeth Swisher*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	*Dr. Swisher supervised all aspects of the project.*
Funding Support:	

Name:	*Maria Harrell PhD*
Project Role:	*Senior scientist*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	12
Contribution to Project:	*Dr. Harrell performed next generation and Sanger Sequencing and DNA extractions*
Funding Support:	

Name:	Barbara Norquist
Project Role:	*Co-investigator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	*Dr. Norquist participated in sequencing and all data analyses and manuscript preparation.*
Funding Support:	Liz Tilberis Career Development Award, OCRF

What other organizations were involved as partners? Nothing to report

APPENDIX

1. JAMA Oncology manuscript, Norquist et al
2. SGO 2016 abstract, Norquist et al

Original Investigation

Inherited Mutations in Women With Ovarian Carcinoma

Barbara M. Norquist, MD; Maria I. Harrell, PhD; Mark F. Brady, PhD; Tom Walsh, PhD; Ming K. Lee, PhD; Suleyman Gulsuner, MD, PhD; Sarah S. Bernards, BS; Silvia Casadei, PhD; Qian Yi, PhD; Robert A. Burger, MD; John K. Chan, MD; Susan A. Davidson, MD; Robert S. Mannel, MD; Paul A. DiSilvestro, MD; Heather A. Lankes, PhD; Nilsa C. Ramirez, MD; Mary Claire King, PhD; Elizabeth M. Swisher, MD; Michael J. Birrer, MD, PhD

Invited Commentary

Supplemental content at jamaoncology.com

IMPORTANCE Germline mutations in *BRCA1* and *BRCA2* are relatively common in women with ovarian, fallopian tube, and peritoneal carcinoma (OC) causing a greatly increased lifetime risk of these cancers, but the frequency and relevance of inherited mutations in other genes is less well characterized.

OBJECTIVE To determine the frequency and importance of germline mutations in cancer-associated genes in OC.

DESIGN, SETTING, AND PARTICIPANTS A study population of 1915 woman with OC and available germline DNA were identified from the University of Washington (UW) gynecologic tissue bank (n = 570) and from Gynecologic Oncology Group (GOG) phase III clinical trials 218 (n = 788) and 262 (n = 557). Patients were enrolled at diagnosis and were not selected for age or family history. Germline DNA was sequenced from women with OC using a targeted capture and multiplex sequencing assay.

MAIN OUTCOMES AND MEASURES Mutation frequencies in OC were compared with the National Heart, Lung, and Blood Institute GO Exome Sequencing Project (ESP) and the Exome Aggregation Consortium (ExAC). Clinical characteristics and survival were assessed by mutation status.

RESULTS Overall, the median (range) age at diagnosis was 60 (28-91) years in patients recruited from UW and 61 (23-87) years in patients recruited from the GOG trials. A higher number of black women were recruited from the GOG trials (4.3% vs 1.4%; $P = .009$); but in patients recruited from UW, there was a higher proportion of fallopian tube carcinomas (13.3% vs 5.7%; $P < .001$); stage I and II disease (14.6% vs 0% [GOG trials were restricted to advanced-stage cancer]); and nonserous carcinomas (29.9% vs 13.1%, $P < .001$). Of 1915 patients, 280 (15%) had mutations in *BRCA1* (n = 182), or *BRCA2* (n = 98), and 8 (0.4%) had mutations in DNA mismatch repair genes. Mutations in *BRIP1* (n = 26), *RAD51C* (n = 11), *RAD51D* (n = 11), *PALB2* (n = 12), and *BARD1* (n = 4) were significantly more common in patients with OC than in the ESP or ExAC, present in 3.3%. Race, histologic subtype, and disease site were not predictive of mutation frequency. Patients with a *BRCA2* mutation from the GOG trials had longer progression-free survival (hazard ratio [HR], 0.60; 95% CI, 0.45-0.79; $P < .001$) and overall survival (HR, 0.39; 95% CI, 0.25-0.60; $P < .001$) compared with those without mutations.

CONCLUSIONS AND RELEVANCE Of 1915 patients with OC, 347 (18%) carried pathogenic germline mutations in genes associated with OC risk. *PALB2* and *BARD1* are suspected OC genes and together with established OC genes (*BRCA1, BRCA2, BRIP1, RAD51C, RAD51D, MSH2, MLH1, PMS2,* and *MSH6*) bring the total number of genes suspected to cause hereditary OC to 11.

Author Affiliations: Author affiliations are listed at the end of this article.

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JAMA Oncol. doi:10.1001/jamaoncol.2015.5495
Published online December 30, 2015.

Ovarian carcinoma (OC) remains the deadliest gynecologic malignancy.[1] Identifying genetic predisposition offers opportunities for cancer prevention. According to previous studies,[2-4] 13% to 18% of OC is associated with germline mutations in *BRCA1* and *BRCA2*. The lifetime risk of developing OC for a woman with a *BRCA2* mutation is approximately 20% and approximately 50% for a *BRCA1* mutation,[5] and risk-reducing salpingo-oophorectomy has been shown to significantly reduce the risk of OC and all-cause mortality.[6,7] Other genes from the *BRCA*-Fanconi anemia pathway such as *BRIP1*, *RAD51C*, and *RAD51D* have been implicated in hereditary OC.[8-13] We previously reported the frequency of these mutations in a small set of unselected patients with OC (n = 360).[4] Several publications[12-14] reporting on a recent series of unselected OC found lower rates of mutations in all OC genes, perhaps secondary to different sequencing methods. Mutations in other genes within this pathway (*PALB2*, *BARD1*, *NBN*, and *CHEK2*, among others) have been identified in patients with OC, but it is unknown if these mutations confer an elevated risk of OC.[4]

We sought to determine the frequency of mutations in known or suspected OC susceptibility genes in a large unselected group of patients with ovarian, peritoneal, and fallopian tube carcinoma (collectively, OC) using a comprehensive targeted sequencing method.

At a Glance

- The purpose of this study was to determine the frequency and importance of inherited mutations in cancer-associated genes in women with ovarian cancer (OC), unselected by age or family history.
- Of 1915 OC patients, 347 (18%) carried a germline mutation in a gene associated with OC risk.
- Mutations in *BRCA1*, *BRCA2*, *BRIP1*, *RAD51C*, *RAD51D*, *PALB2*, and *BARD1* were more frequent in patients with OC than in the population.
- Mutations were found in all OC histologic subtypes with the exception of mucinous histology, suggesting that genetic testing is warranted in all nonmucinous histologies.
- Mutation status influenced both progression-free and overall survival in OC clinical trials and should be considered when interpreting results and designing future trials.

Methods

Patients with OC were identified from 3 sources: (1) patients undergoing primary treatment at the University of Washington (UW) Medical Center, and (2) patients who consented for translational research with available DNA from Gynecologic Oncology Group (GOG) protocol 218, and (3) GOG protocol 262. Patients were enrolled at diagnosis and were not selected for age or family history. Pathology was centrally reviewed by gynecologic pathologists, and unsure cases were resolved by consensus. GOG-218 and GOG-262 were large randomized phase III trials studying primary advanced-stage OC. A subset of the UW patients has been previously described.[4] All patients provided written informed consent on protocols approved by an institutional review board.

Germline DNA extracted from blood was sequenced using BROCA, a targeted capture, massively parallel sequencing test developed at the University of Washington.[15] For this study, we sequenced *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CHEK2*, *FAM175A*, *FANCP*, *MLH1*, *MSH2*, *MSH6*, *MRE11A*, *NBN*, *PALB2*, *PMS2*, *PTEN*, *RAD50*, *RAD51C*, *RAD51D*, and *TP53*. Sequencing reads were aligned to the human reference genome (hg19). Variants were identified using GATK37 and Pindel after indel realignment and base quality recalibration. Variants including copy number variations (CNVs) were detected as previously described.[15-17] Missense mutations were only included if proven to be damaging (eg, *BRCA1* C61G[18]). *TP53* missense mutations were classified as deleterious based on available functional data as per the International Agency for Research on Cancer.[19]

Two publically available, overlapping, online exome sequencing data sets were used to estimate population mutation frequencies: the European American (EA) data set from the National, Heart, Lung, and Blood Institute Exome Sequencing Project (ESP)[20] and the Exome Aggregation Consortium (ExAC).[21] To account for inaccuracy in indel calling in the ESP, we visually inspected the read data for all coding indel calls in the ESP data set for the genes of interest. Calls that were low quality (<17 Phred score) and low coverage (<5) were excluded. The EA group was used because most OC subjects were white, and the total ESP population overrepresents African Americans. ExAC mutation frequencies were weighted to match the racial distribution in OC cases. Because these exome databases do not include CNVs, CNVs were removed from the OC frequencies for this comparison. Splice mutations were also excluded from OC and population mutation frequencies because they could not all be verified to be damaging.

Clinical information was collected as per the GOG 218 and 262 protocols or extracted from the medical records for UW patients. Race and ethnicity (self-reported in the GOG patients and as noted in the medical records for UW patients) were assessed to determine if they affected mutation rates.

Contingency tables were analyzed with χ^2 or Fisher exact tests. In GOG patients, progression-free survival (PFS) and overall survival (OS) were defined as the time between enrollment and progression[22] or death, respectively. Proportional hazards models were used to provide estimates of relative hazards adjusted for clinical characteristics (further description, as well as the log files of the statistical analyses, are available in the Supplement). The Wald test was used to assess the null hypotheses of equal hazards. All *P* values are 2-sided.

Results

Description of Study Population

Clinical characteristics are provided in **Table 1**. The median (range) age at diagnosis was 60 (28-91) years for UW patients and 61 (2387) years for GOG patients. There was an increased fraction of black women recruited from the GOG trials (4.3% vs 1.4%; $P = .009$); but in patients recruited from UW, there was a higher proportion of fallopian tube carcinomas (13.3% vs 5.7%; $P < .001$); stage I and II disease (14.6% vs 0% [GOG trials

Table 1. Clinical Characteristics of Ovarian Cancer Patients

Characteristic	No. (%) GOG 218 and 262	UW
No.	1345	570
Age, y		
<40	35 (2.6)	16 (2.8)
40-49	176 (13.1)	90 (15.8)
50-59	426 (31.7)	165 (28.9)
60-69	451 (33.5)	172 (30.2)
70-79	229 (17.0)	92 (16.1)
≥80	28 (2.1)	35 (6.1)
Race/ethnicity		
Non-Hispanic white	1176 (87.4)	505 (88.6)
Hispanic	53 (4.3)	16 (2.8)
Non-Hispanic black	58 (4.3)	8 (1.4)
Asian/Pacific Islander	27 (2.0)	11 (1.9)
Other or Unknown	31 (2.3)	30 (5.3)
Disease site		
Ovary	1076 (80.0)	426 (74.7)
Peritoneal	192 (14.3)	57 (10.0)
Fallopian tube	77 (5.7)	76 (13.3)
Ovary/endometrial	0	11 (1.9)
Stage[a]		
I	0	41 (7.2)
II	0	42 (7.4)
III	977 (72.6)	380 (66.7)
IV	365 (27.1)	107 (18.8)
Histology		
Serous		
High-grade	1118 (83.1)	380 (66.7)
Low-grade	51 (3.8)	19 (3.3)
Carcinoma, NOS	84 (6.2)	81 (14.2)
Endometrioid		
Low-grade	4 (0.3)	9 (1.6)
High-grade	38 (2.8)	26 (4.6)
Clear cell	30 (2.2)	28 (4.9)
Carcinosarcoma	3 (0.2)	19 (3.3)
Mucinous	9 (0.7)	7 (1.2)
Transitional cell	8 (0.6)	1 (0.2)

Abbreviations: GOG, Gynecologic Oncology Group; NOS, not otherwise specified; UW, University of Washington.

[a] Stage was not available for 3 GOG patients.

were restricted to advanced-stage cancer]); and nonserous carcinomas (29.9% vs 13.1%, $P < .001$).

Frequency of Mutations in *BRCA1*, *BRCA2*, and Mismatch-Repair Genes

Of 1915 patients, 182 (9.5%) had mutations in *BRCA1* and 98 (5.1%) had mutations in *BRCA2*. Of *BRCA1* and *BRCA2* mutations, 38 of 280 (13.6%) were in Ashkenazi Jewish founder mutations *BRCA1* 185delAG (c.68_69delAG) (n = 18) and 5382insC (c.5266dupC) (n = 14) and *BRCA2* 6174delT (c.5946delT) (n = 6). Overall, 16 of 182 (8.8%) *BRCA1* mutations were large genomic duplications or deletions, also called copy number variants (CNVs). Eight of 1915 patients (0.4%) carried mutations in genes involved in mismatch repair (MMR), including 4 in *PMS2*, 3 in *MSH6*, and 1 in *MLH1*. There were no mutations found in *MSH2*. Mutation frequencies for *BRCA1*, *BRCA2*, and MMR did not differ by ascertainment. Median coverage was 219 fold.

Mutations in Other Genes and Comparison With Population Frequency

Mutations in *BRCA1*, *BRCA2*, *BRIP1*, *PALB2*, *RAD51C*, *RAD51D*, and *BARD1* were all significantly more common in women with OC compared with the ESP or ExAC (**Table 2**), and using these data, we categorized these genes together with the MMR genes as OC-associated genes. Other putative cancer-associated genes such as *CHEK2*, *NBN*, *RAD50*, *FAM175A*, and *MRE11A* were not more frequently mutated in women with OC. Deleterious mutations in *ATM* and *TP53* were more frequent in OC patients when compared with ExAC, but this was not significant when compared with the ESP. Odds ratios for OC are presented in Table 2. There were no mutations found in *PTEN* or *FANCP*.

In total, 347 women with OC (18.1%) had 352 mutations in OC-associated genes: 280 (14.6%) in *BRCA1* or *BRCA2*, 64 (3.3%) in another BRCA-Fanconi anemia OC-associated gene (*BRIP1*, *PALB2*, *RAD51C*, *RAD51D*, or *BARD1*), and 8 (0.4%) in an MMR gene. Five patients (1.4%) of 347 had more than 1 mutation. Individual mutations and associated clinical data for OC-associated genes other than *BRCA1* and *BRCA2* are provided in the eTable in the Supplement.

Clinical Characteristics and Mutation Status

Ovarian cancer–associated mutations were separated into 5 categories: (1) *BRCA1*, (2) *BRCA2*, (3) other BRCA-Fanconi anemia OC-associated (*BRIP1*, *PALB2*, *RAD51C*, *RAD51D*, *BARD1*), (4) mismatch repair (*MSH6*, *PMS2*, *MSH2*, *MLH1*), and (5) and no mutation (either a mutation in a gene not clearly associated with OC or no mutation). Women with *BRCA1* mutations had a median (range) age at OC onset of 52 (27-77) years, while the median (range) age at OC onset for those with no mutation was 62 (23-91) years ($P < .001$). The median (range) age of patients with a *BRCA2* mutation was 59 (41-83) years; BRCA-Fanconi anemia OC-associated mutation, 60 (34-79) years; and MMR gene mutations, 50 (47-69) years. **Table 3** summarizes clinical information by each OC-associated gene. In the 54 of 570 (9.5%) UW patients with a previous history of breast cancer, 28 of 54 (51.8%) had mutations in OC-associated genes, 17 (31.5%) in *BRCA1*, 9 (16.7%) in *BRCA2*, and 2 (3.7%) in other OC-associated genes (1, *BRIP1* and 1, *RAD51D*).

Figure 1 summarizes mutation frequency by clinical characteristics. Disease site, race, and ethnicity were not associated with mutation frequency. Overall, 294 patients with high-grade (grade 2-3) serous carcinomas had OC-associated gene mutations, an overall mutation frequency of 19.6% and a combined *BRCA1* and *BRCA2* frequency of 16.1%. Patients with unspecified carcinomas, endometrioid, carcinosarcoma, and transitional cell histology had similar mutation rates to high-grade serous histology, both for all OC genes and for *BRCA1* and *BRCA2*. Patients with clear cell histology had a lower overall mutation frequency compared with high-grade serous cases

Table 2. Mutation Frequencies in Ovarian Cancer Cases Compared With Population

Gene	Mutation (Freq) Cases (n = 1915)	Minus CNVs/Splice (n = 1915)	Adjusted ESP EA (n = 4300)	Cases vs ESP EA, OR (95% CI)	P Value	Mutation (Frequency) ExAC (n = 36 276)[a]	Cases vs ExAC, OR (95% CI)	P Value
Genes More Frequently Mutated in Ovarian Cancer								
BRCA1	182 (0.0950)	160 (0.0836)	8 (0.0019)	48.9 (24.0-100)	<.001	114 (0.0031)	29.0 (22.7-37.1)	<.001
BRCA2	98 (0.0512)	95 (0.0496)	16 (0.0037)	14.0 (8.2-23.8)	<.001	149 (0.0041)	12.7 (9.7-16.4)	<.001
BRIP1	26 (0.0136)	20 (0.0104)	5 (0.0012)	9.1 (3.4-24.2)	<.001	60 (0.0017)	6.4 (3.8-10.6)	<.001
PALB2	12 (0.0062)	9 (0.0047)	2 (0.0005)	10.2 (2.2-47.0)	<.001	39 (0.0011)	4.4 (2.1-9.1)	<.001
RAD51C	11 (0.0057)	7 (0.0037)	1 (0.0002)	15.8 (1.9-128)	.002	39 (0.0011)	3.4 (1.5-7.6)	.005
RAD51D	11 (0.0057)	8 (0.0042)	2 (0.0005)	9.0 (1.9-42.5)	.002	14 (0.0004)	10.9 (4.6-26.0)	<.001
BARD1	4 (0.0021)	4 (0.0021)	0	20.3 (1.1-377)	.009	18 (0.0005)	4.2 (1.4-12.5)	.02
Other Known or Suspected Cancer Associated Genes								
CHEK2	11 (0.0057)	7 (0.0036)	25 (0.0058)	0.6 (0.3-1.5)	.37	297 (0.0082)	0.4 (0.2-0.9)	.04
ATM	11 (0.0057)	10 (0.0052)	9 (0.0021)	2.5 (1.0-6.2)	.07	79 (0.0022)	2.4 (1.2-4.7)	.01
NBN	9 (0.0047)	6 (0.0031)	6 (0.0014)	2.2 (0.7-7.0)	.26	49 (0.0014)	2.3 (0.99-5.4)	.09
TP53	6 (0.0031)	6 (0.0031)	4 (0.0009)	3.4 (0.95-12.0)	.08	39 (0.0011)	2.9 (1.2-6.9)	.03
RAD50	3 (0.0016)	3 (0.0016)	11 (0.0026)	0.6 (0.2-2.2)	.57	87 (0.0024)	0.7 (0.2-2.1)	.63
FAM175A	3 (0.0016)	3 (0.0016)	5 (0.0012)	1.3 (0.3-5.6)	.71	30 (0.0008)	1.9 (0.6-6.2)	.23
MRE11A	2 (0.0010)	1 (0.0005)	1 (0.0002)	2.2 (0.1-36.0)	.52	25 (0.0007)	0.8 (0.1-5.6)	>.99

Abbreviations: CNV, copy number variant; EA, the European American; ExAC, Exome Aggregation Consortium; ESP, Exome Sequencing Project; OR, odds ratio.

[a] Mutation numbers in ExAC are adjusted for racial groups using weighted frequencies.

Table 3. Clinical Characteristics by Ovarian Cancer-Associated Gene[a]

Gene	No.	Age, Median (range)	Stage[b] I	II	III	IV	Histology HGS	LGS	HGE	LGE	Carc	CC	CS	M	T
BRCA1	182	52 (27-77)	1	4	130	47	155	3	4	0	14	4	1		1
BRCA2	98	59 (41-83)	0	1	75	22	85	1	3	0	9	0	0	0	0
BRIP1	26	65.5 (43-79)	1	0	12	13	22	0	0	1	3	0	0	0	0
PALB2	12	56 (49-65)	0	0	10	2	9	0	0	0	2	1	0	0	0
RAD51C	11	64 (47-70)	0	1	7	3	7	0	1	0	2	0	1	0	0
RAD51D	11	54 (34-75)	1	0	5	5	7	0	1	0	3	0	0	0	0
BARD1	4	55.5 (53-60)	0	1	2	1	3	0	0	0	1	0	0	0	0
PMS2	4	52.5 (48-69)	0	0	2	2	4	0	0	0	0	0	0	0	0
MSH6	3	49 (47-62)	2		1	0	1	0	0	2	0	0	0	0	0
MLH1	1	47	0	0	0	1	0	0	0	0	1	0	0	0	0
None	1568	62 (23-91)	37	35	1117	376	1208	66	55	11	131	53	20	16	8

Abbreviations: Carc, unspecified carcinoma; CS, carcinosarcoma; CC, clear cell; HGE, high-grade, grade 2-3, endometrioid; HGS, high-grade, grade 2-3, serous; LGE, low-grade, grade 1, endometrioid; LGS, low-grade, grade 1, serous; M, mucinous; T, transitional cell.

[a] If a patient demonstrated more than 1 mutation (n = 5), each gene mutated is listed.

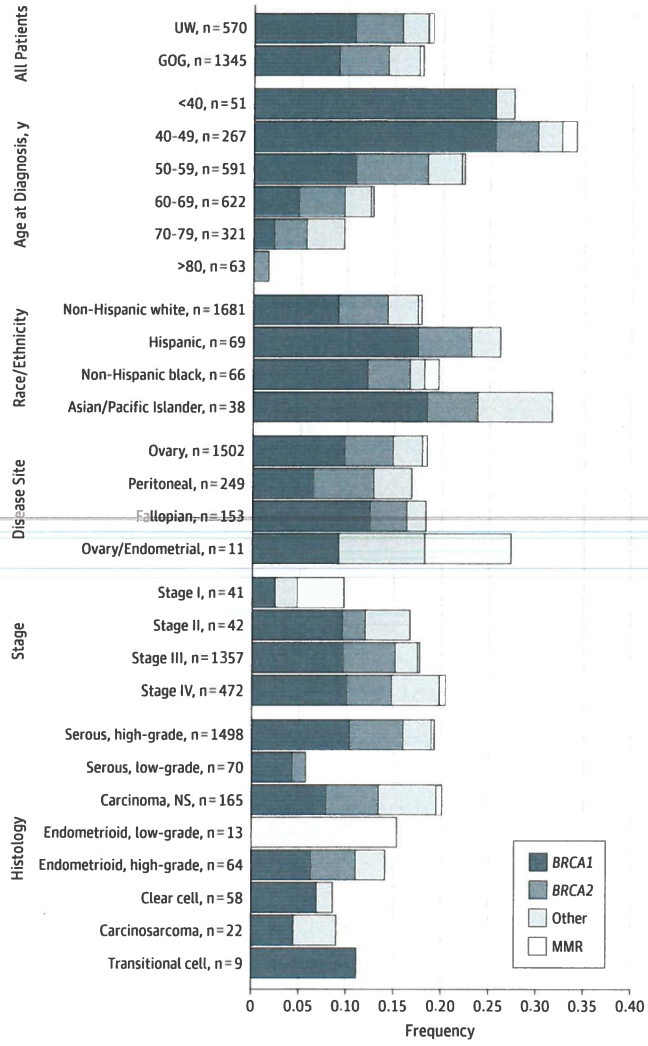
[b] Stage was not available for 3 GOG patients.

(8.6% vs 19.6%; $P = .04$), but differences in the *BRCA1* and *BRCA2* mutation frequency were not significant (6.9% vs 16.1%; $P = .07$). In patients with low-grade (grade 1) serous histology compared with high-grade serous cases, there were fewer mutations overall (5.7% vs 19.6%; $P = .003$) and for *BRCA1* and *BRCA2* (5.7% vs 16.1%; $P = .02$). In 16 women with mucinous histology, no OC-associated mutations were found.

Survival

In the GOG patients, hazard ratios (HRs) for progression and death were adjusted for protocol, study treatment, stage, residual disease, and initial performance status. The GOG patients were followed for 5 years. The median PFS for GOG patients without a *BRCA2* mutation was 21.9 months, and the median PFS for those with no mutation was 14.2 months (HR, 0.60; 95% CI, 0.45-0.79; $P < .001$). The median OS for GOG patients with a *BRCA2* mutation had not yet been reached, and the median OS for GOG patients with a *BRCA2* mutation was 44 months (HR, 0.39; 95% CI, 0.25-0.60; $P < .001$) (**Figure 2**). The median OS in patients with a *BRCA1* mutation was 56 months and was intermediate between patients with a *BRCA2* mutation and women without mutations. Relative to patients without mutations, the HR for *BRCA1* mutations was 0.75 (95% CI, 0.56-1.00; $P = .05$) (Figure 2B). In the UW group, OS

Figure 1. Mutation Status by Clinical Characteristics in Patient With OC



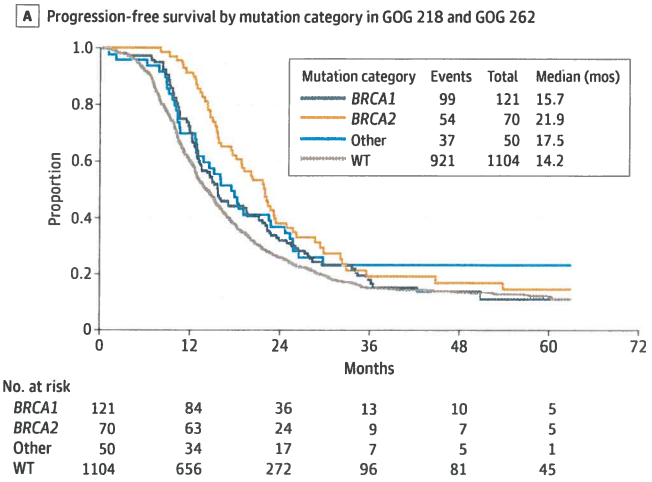
Sixty-one patients had unknown or other race/ethnicity and are not shown, and 3 Gynecologic Oncology Group patients had no listed stage. In the histology section, 16 mucinous (all no mutation) are not shown. GOG indicates Gynecologic Oncology Group; Other indicates the genes *BRIP1*, *PALB2*, *RAD51C*, *RAD51D*, and *BARD1*; MMR indicates mismatch repair genes *PMS2*, *MSH6*, and *MLH1*; NS indicates nonsquamous, OC, ovarian carcinoma; UW indicates University of Washington.

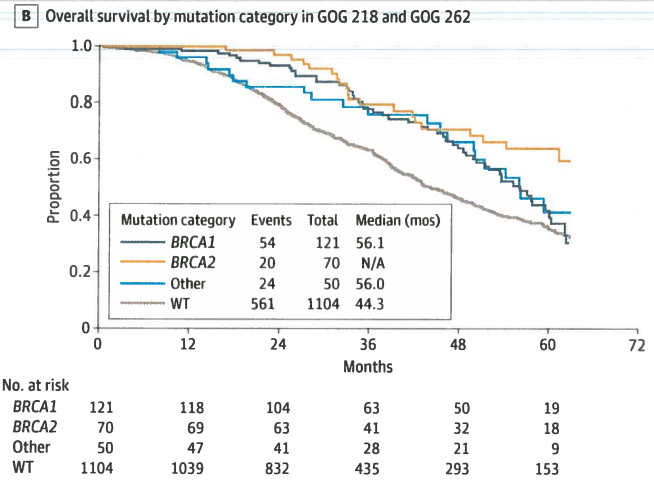
for women with stage-III and stage-IV OC was longer in both patients with a *BRCA2* mutation (median, 70 months; $P = .004$, log-rank) and *BRCA1* mutation (median, 46 months; $P = .04$, log-rank) when compared with women without mutations (median, 36 months). Exploratory analyses of the individuals from the GOG studies suggest that compared with those without mutations, the PFS event rates for those individuals with *BRCA1* ($P = .009$) or *BRCA2* ($P < .001$) mutations are time-dependent (Figure 2A). Specifically, the PFS event rate is initially lower for those with *BRCA1* or *BRCA2* mutations but this advantage declines over time. There is a similar time-dependent decline in the relative death rate for those with *BRCA1* mutations compared with those without mutations ($P < .001$) (Figure 2B). In both GOG (Figure 2) and UW patients (data not shown), survival was similar for women with mutations in *BRCA1* and other OC-associated genes in the BRCA-Fanconi anemia pathway, but the analysis was limited due to low non-*BRCA* mutation frequency.

Discussion

BRCA1 and *BRCA2* are part of the BRCA-Fanconi anemia DNA repair pathway that controls DNA repair via homologous recombination.[23-25] It is plausible that damaging mutations in other genes in this pathway may also confer a risk of OC. Indeed, our data demonstrate that mutations in the BRCA-Fanconi anemia OC-associated genes *BRIP1*, *RAD51C*, and *RAD51D* together account for an additional 2.5% of unselected OC. Previous studies of high-risk families or specific founder mutations have suggested that damaging mutations in *BRIP1*, *RAD51C*, and *RAD51D* confer a relative risk increase for OC of 6 to 8 fold and an absolute lifetime risk of 10% to 15%.[8-11,26,27] Our data from unselected patients with OC confirm *BRIP1*, *RAD51C*, and *RAD51D* as important OC-associated genes. The Fanconi anemia gene *BRIP1 (FANCJ)*, mutated in 1.4% of women with OC, is the next most commonly mutated gene in OC after *BRCA1* and *BRCA2*.

Figure 2. Survival by Mutation Category in the GOG 218 and GOG 262 Trials





A, Progression-free survival by mutation category in GOG 218 and GOG 262. B, Overall survival by mutation category in GOG 218 and GOG 262. GOG indication Gynecologic Oncology Group; NA indicates not applicable; Other indicates the genes *BRIP1*, *PALB2*, *RAD51C*, *RAD51D*, and *BARD1*; WT indicates wild type.

In addition to identifying mutations in genes already implicated in OC, we identified mutations in the *BRCA*-Fanconi anemia genes *PALB2* and *BARD1* more frequently in women with OC compared with population frequencies in the ESP and ExAC (Table 2). *PALB2* (*FANCN*) is a Fanconi anemia gene whose protein binds BRCA1 and BRCA2 at sites of DNA damage.[28] Mutations in *PALB2* are associated with an elevated risk of breast cancer and have been identified in families with both breast cancer and OC[29-31] but have not been clearly associated with OC risk.[28,29] An analysis of germline mutations in high-grade serous OC from the Cancer Genome Atlas project identified *PALB2* as the only gene other than *BRCA1* and *BRCA2* to be significantly more frequently mutated compared with a subset of ESP controls from the Women's Health Initiative. The *PALB2* mutation frequency in our larger series was significant compared with population rates and was associated with similar odds ratios for OC as *RAD51C*, *RAD51D*, and *BRIP1* (Table 2). The corrected frequency of *PALB2* mutations in the ESP is consistent with *PALB2* mutation rates from other previously published control sets[30,32] where full sequencing of *PALB2* was performed supporting the use of the ESP as a comparison population.

Three recent publications[12-14] by the Ovarian Cancer Association Consortium (OCAC) have examined rates of mutations in *BRCA1*, *BRCA2*, MMR genes, *RAD51C*, *RAD51D*, *BRIP1*, *PALB2*, and *BARD1* compared with controls. The sequencing methodology reported by OCAC is different from our study. As the authors acknowledged, their sequencing methods resulted in relatively low coverage, were unable to detect genomic rearrangements, and were likely to lead to underestimation of mutation frequencies.[12] A recent OCAC study[14] demonstrates a less sensitive sequencing method with a *BRCA1* mutation rate of 3.8% that is significantly lower than our *BRCA1* mutation rate of 9.5% (84 of 2222 vs 182 of 1915; $P < .001$, Fisher exact test) and lower than that reported in other population-based series.[2,3] Even if we exclude genomic rearrangements, which OCAC could not detect, our 8.7% *BRCA1* mutation rate remains significantly higher (166 of 1915 vs 84 of 2222; $P < .001$, Fisher exact test). Despite a mutation rate for *RAD51C* and *RAD51D* that was approxi-

mately 30% lower than ours, OCAC identified an odds ratio similar to our calculation for these genes. OCAC did not find a difference in mutation frequency for *PALB2* or *BARD1* in OC cases vs controls,[13] but their lower mutation rates in cases and an unexpectedly high *PALB2* mutation rate in controls affected their power to detect such a difference.

BARD1 forms a heterodimer with BRCA1 mediated by their homologous ring finger motifs.[33] This heterodimerization is critical to several tumor suppressor functions of BRCA1, and mutations in *BRCA1* that affect binding to BARD1 are associated with increased cancer risk.[34] *BARD1* mutations were rare in OC patients but significantly more common than in the general population, leading to wide confidence intervals for the generated odds ratio for OC (Table 2). However, the significant *P* value and the shared homology to *BRCA1* support *BARD1* as a rare OC susceptibility gene. These results are interpreted with some caution as 2 of the *BARD1* mutation carriers also had mutations in *BRCA1* (eTable in the Supplement). Mutations in *BARD1* have also been identified in women with triple negative breast cancer, another phenotype associated with *BRCA1* mutations.[35] Additional studies are needed to define the absolute risk of *BARD1* for both breast cancer and OC. The addition of *BARD1* and *PALB2* to other known OC-associated genes (*BRCA1*, *BRCA2*, *RAD51C*, *RAD51D*, *BRIP1*, *PALB2*, *MSH2*, *MLH1*, *MSH6*, and *PMS2*) brings the total suspected hereditary OC genes to 11.

In contrast to *PALB2* and *BARD1*, mutations in other genes in the BRCA-Fanconi anemia pathway previously implicated in breast cancer risk but not OC risk (*CHEK2*, *NBN*, *RAD50*, *FAM175A*, and *MRE11A*) were not more commonly mutated in patients with OC. Mutations in *TP53* that causes early onset breast cancer and Li Fraumeni syndrome and mutations in *ATM* were slightly more frequent in OC than in population controls, which appeared significant when compared with the larger ExAC database. Given that mutations in all of these genes are rare, confidence intervals are wide, and we cannot fully exclude that some of these genes, in particular *ATM* and *TP53*, are associated with some risk for OC. However, a relatively high risk of OC is unlikely.

Mutations in MMR genes (*MSH2*, *MLH1*, *MSH6*, and *PMS2*), which cause Lynch syndrome are often cited as the other major cause of hereditary OC in addition to *BRCA1* and *BRCA2*.[36] However, MMR mutations were infrequent (n = 8; 0.4%) in this series. Interestingly, 7 of 8 (88%) MMR mutations in women with OC occurred in *PMS2* or *MSH6*, in contrast to patients with colon cancer, in which *MSH2* and *MLH1* mutations predominate.[37] Notably, 2 of 3 OCs with *MSH6* mutations were endometrioid and low stage, but all 4 *PMS2*-mutated cases were advanced-stage high-grade serous carcinomas. Our data are consistent with a population-based study[38] of 1638 women with invasive OC who were sequenced for mutations in *MLH1*, *MSH2*, and *MSH6*, where 9 of 1638 (0.5%) had mutations (5 of 9 in *MSH6* [*PMS2* not assessed]).

The National Comprehensive Cancer Network recommends genetic testing for all women affected by OC, but some authors have proposed limiting *BRCA1* and *BRCA2* testing to high-grade serous OC and suggested that *BRCA1* and *BRCA2* mutations found in nonserous cases represent pathological misclassification.[39] In our study, all cases were centrally reviewed by a panel of gynecologic pathologists, minimizing pathological misclassification. The overall mutation rate for high-grade serous histology was not significantly different from the mutation frequency in undifferentiated carcinoma, endometrioid, or carcinosarcoma histologies. While clear cell and low-grade serous OC did have fewer mutations compared with high-grade serous OC, the mutation rates (5 of 58 patients [8.6%] and 4 of 70 patients [5.7%]) are probably still high enough to warrant genetic testing. Therefore, our data do not support the restriction of genetic testing to women with high-grade serous OC. Race and ethnicity also did not predict mutation status and should not be used to guide testing. Consistent with previous studies, OC survival was correlated with mutation status, with *BRCA2* mutation carriers having the longest PFS and OS.[2,40] This study is unique in that cancer treatments were standardized within the confines of clinical trials, reducing potential bias in how mutation status affected survival. Mutation status should be considered when analyzing outcomes of OC clinical trials.

There are currently no published guidelines for managing unaffected women found to have mutations in *BRIP1*, *PALB2*, *RAD51C*, *RAD51D*, and *BARD1*. We found similar odds ratios for OC conferred by damaging mutations in these genes to the previously identified 6- to 8- fold relative risk in *BRIP1*, *RAD51C*, and *RAD51D*[8-11,26,27] and a similar age distribution of OC diagnosis for women with mutations in these genes compared with *BRCA2* (Table 2) (Table 3). If a lifetime risk of 10% to 15% is confirmed for these genes, it would be reasonable to consider a risk-reducing salpingo-oophorectomy by age 45 years.

Limitations

There are several limitations to this study. This was not a population-based study, and the GOG cases were limited to stage-III andstage-IV cancers. However, all patients were enrolled at the time of diagnosis, which reduces survival bias, and all patients were also unselected for age or family history. The ESP is thought to reflect US population mutation rates as opposed to cancer-free controls because cancer status is not fully characterized. ExAC has the advantage of sequencing data on a large number of people characterized by racial and ethnic group, but ExAC also includes patients with known malignancies such as those in the Cancer Genome Atlas studies. The inclusion of known patients with cancer in ExAC could falsely lower our calculated odds ratio for OC. Ideally, we would use a larger, well-characterized control group with matching age and race and known cancer status. However, it is reassuring that mutation frequencies in the ESP for these genes were similar to previously published controls.[8,9,30,32,41] Further research is needed to clarify the roles of inherited mutations in *PALB2* and *BARD1* in ovarian cancer risk.

Conclusions

We present data from a large, comprehensively sequenced group of unselected OC patients and provide data to implicate 2 new suspected hereditary OC genes, *BARD1* and *PALB2*.

Overall, 347 of 1915 (18.1%) OC patients had 352 germline mutations in 11 OC genes (*BRCA1*, *BRCA2*, *BRIP1*, *RAD51C*, *RAD51D*, *PALB2*, *BARD1*, *PMS2*, *MSH6*, *MLH1*, and *MSH2*). Mutations were identified in all histologies of OC with the exception of mucinous carcinoma, and 72 of 352 (20.5%) OC-associated mutations occurred in a gene other than *BRCA1* or *BRCA2*.

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Study supervision: Norquist, Swisher, Birrer.
Histopathological review of biospecimens and support from the GOG tissue bank: Ramirez.
Interpretation of genetic data: King.

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Additional Information: The following institutions participated in GOG 218 and 262: Roswell Park Cancer Institute, University of Alabama at Birmingham, Duke University Medical Center, Abington Memorial Hospital, Walter Reed Army Medical Center, Wayne State University, University of Minnesota Medical School, Mount Sinai School of Medicine, Northwestern Memorial Hospital, University of Mississippi Medical Center, Colorado Gynecologic Oncology Group PC, University of California at Los Angeles, University of Washington, University of Pennsylvania Cancer Center, Milton S. Hershey Medical Center, University of Cincinnati, University of North Carolina School of Medicine, University of Iowa Hospitals and Clinics, University of Texas Southwestern Medical Center at Dallas, Indiana University School of Medicine, Wake Forest University School of Medicine, University of California Medical Center at Irvine, Rush-Presbyterian-St Luke's Medical Center, Magee Women's Hospital, SUNY Downstate Medical Center, University of Kentucky, University of New Mexico, The Cleveland Clinic Foundation, State University of New York at Stony Brook, Washington University School of Medicine, Memorial Sloan-Kettering Cancer Center, Cooper Hospital/University Medical Center, Columbus Cancer Council, MD Anderson Cancer Center, University of Massachusetts Medical School, Fox Chase Cancer Center, Women's Cancer Center, University of Oklahoma, University of Virginia Health Sciences Center, University of Chicago, Mayo Clinic, Case Western Reserve University, Tampa Bay Cancer Consortium, Yale University, GOG Japan-Saitama Medical University International Medical Center, University of Wisconsin Hospital, Cancer Trials Support Unit, University of Texas - Galveston, Women and Infants Hospital, Korean Gynecologic Oncology Group, The Hospital of Central Connecticut, Georgia Core, GYN Oncology of West Michigan, PLLC, Aurora Women's Pavilion of West Allis Memorial Hospital, Fred Hutchinson Cancer Research Center, UCSF - Mount Zion, St Joseph's Hospital and Medical Center, Carolinas Medical Center/Levine Cancer Institute and Community Clinical Oncology Program.

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ANNUAL MEETING ON WOMEN'S CANCER
SAN DIEGO
MARCH 19–22, 2016

Mutations in Homologous Recombination Genes and Response to Treatment in GOG 218: an NRG Oncology Study

Barbara Norquist, MD
University of Washington, Seattle, WA

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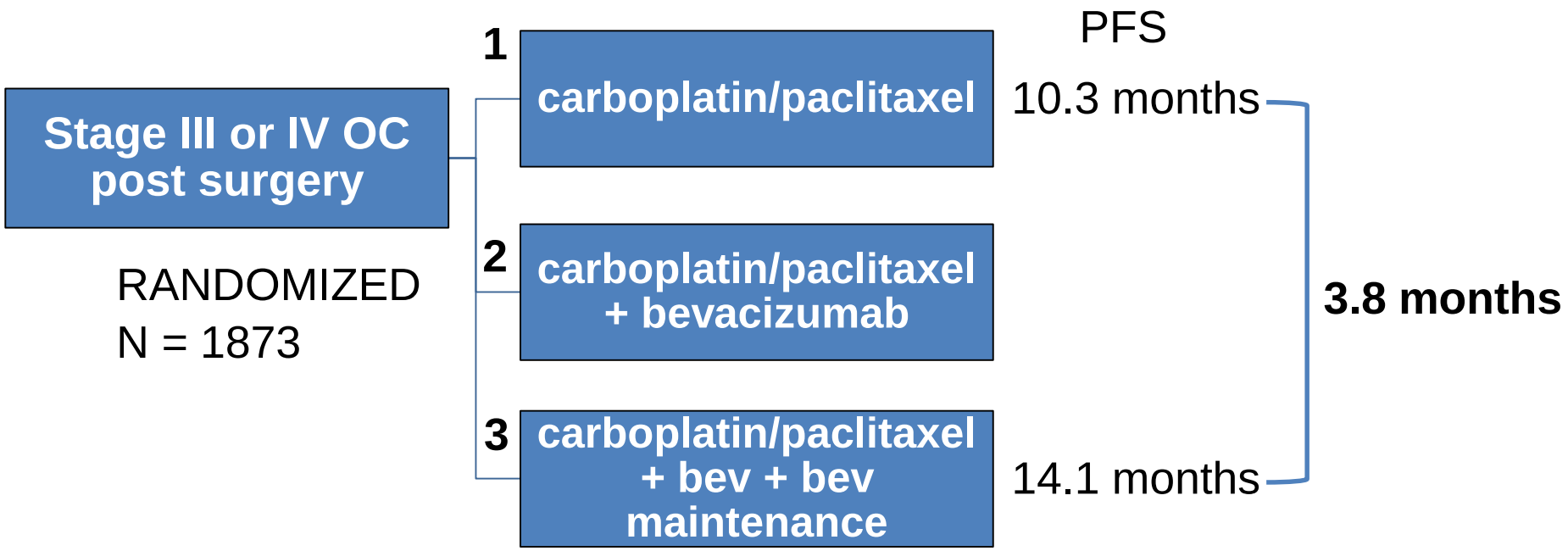
Bringing Together the Best in Women's Cancer Care

VERBAL DISCLOSURE

- I have no disclosures
- Co-author disclosures:
  - Dr. Burger reports participation in advisory boards for Genentech, Gradalis, and Nucana; and participation on data monitoring committees for Janssen and Morphotek
  - Dr. Mannel reports participation in advisory boards for Endocyte, AstraZenica, MedImmune, Oxigene, Advaxis, and Amgen.

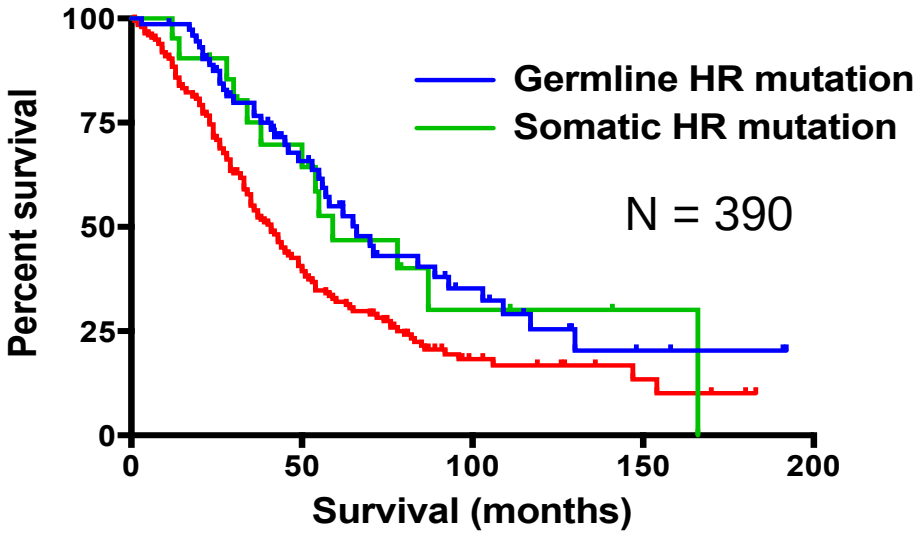
GOG 218 – adding bevacizumab in primary ovarian cancer



Burger et al, NEJM, 2011

Defects in homologous recombination impact prognosis in ovarian cancer

- *BRCA1* and *BRCA2* are homologous recombination genes
- Homologous recombination is the primary way that cells repair double-strand DNA breaks



Median OS (months):	
Germline	66
Somatic	59
No HR mutation	41

Pennington, Clin Ca Res, 2014

Hypothesis

- Ovarian cancer (OC) patients with defective homologous recombination may derive less benefit from bevacizumab

Objective

- To assess the impact of mutations in genes affecting homologous recombination on response to treatment in GOG 218

Study population: 1195 of 1873 (63.8%) sequenced

Characteristic	Sequenced N = 1195	Not Sequenced N = 678	P-Value
Mean Age	59.6 years	60.2 years	0.62
Race:			
Non-Hispanic White	1048 (87.7%)	526 (76.4%)	<0.001
Debulking status:			
Stage III Optimal	465 (38.9%)	175 (25.8%)	<0.001
Stage IV	277 (23.2%)	204 (30.1%)	
Histology:			
Gr 2/3 Serous	971 (81.2%)	526 (77.6%)	0.06
Bev Exposure:			
Arm 3: CT+B->B	401 (33.6%)	222 (32.7%)	0.28

BROCA-HR sequencing

- DNA from blood **and/or** neoplastic tissue
- Sequenced with BROCA-HR, targeted capture, multiplex sequencing assay[1]
- Defects in homologous recombination defined as damaging mutations in *ATM*, *ATR*, *BARD1*, *BLM*, ***BRCA1***, ***BRCA2***, *BRIP1*, *CHEK2*, *MRE11A*, *NBN*, *PALB2*, *RAD51C*, *RAD51D*, *RBBP8*, *SLX4*, and *XRCC2*

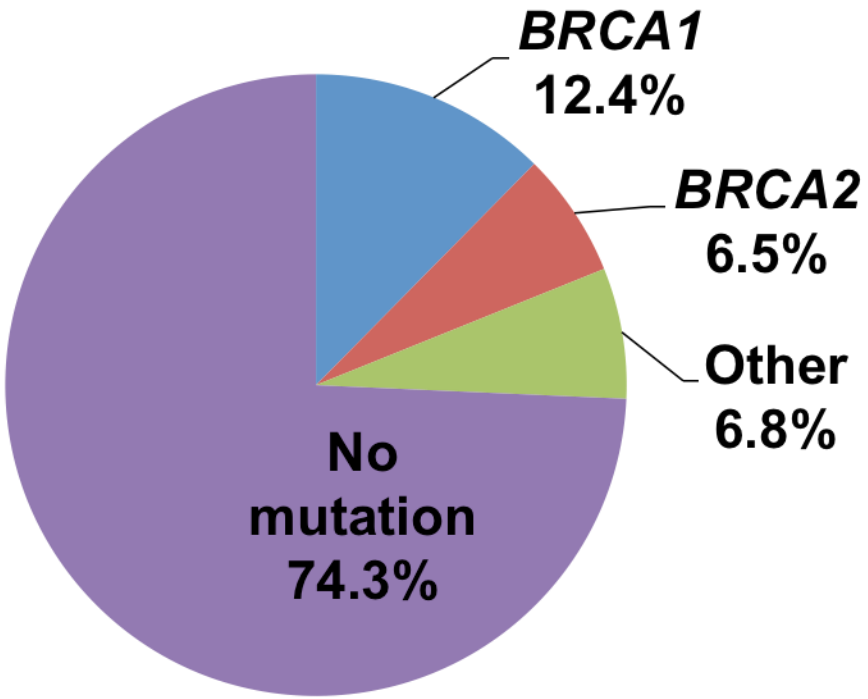
1. Walsh et al, PNAS 2010

Statistics

- Proportional hazards models were used to provide estimates of relative hazards for progression free survival (PFS) and overall survival (OS) by genotype, adjusted for clinical characteristics
- The relationship between mutation status and bevacizumab effect was assessed with a test of interaction

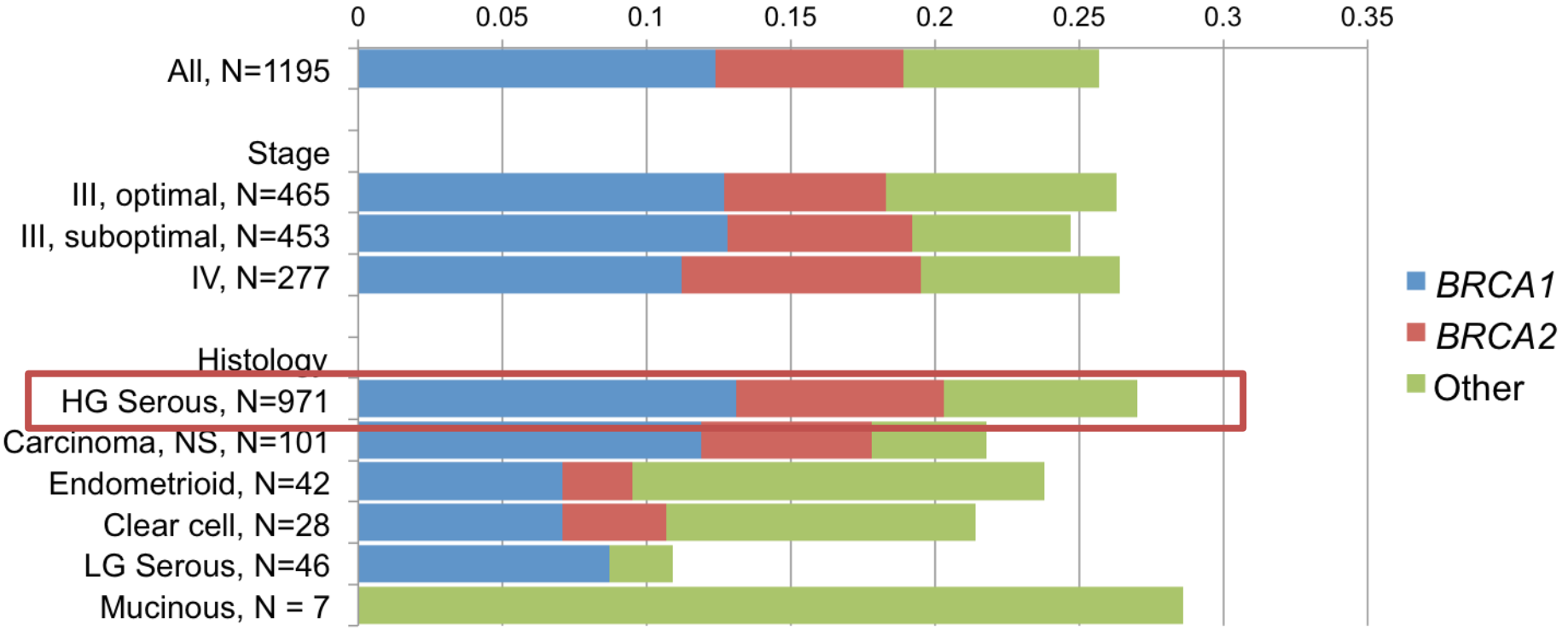
Proportion of OC patients with mutations in homologous recombination genes

Gene	N	
BRCA1	148	
BRCA2	78	
Other	81	
Total	**307**	**(25.7%)**

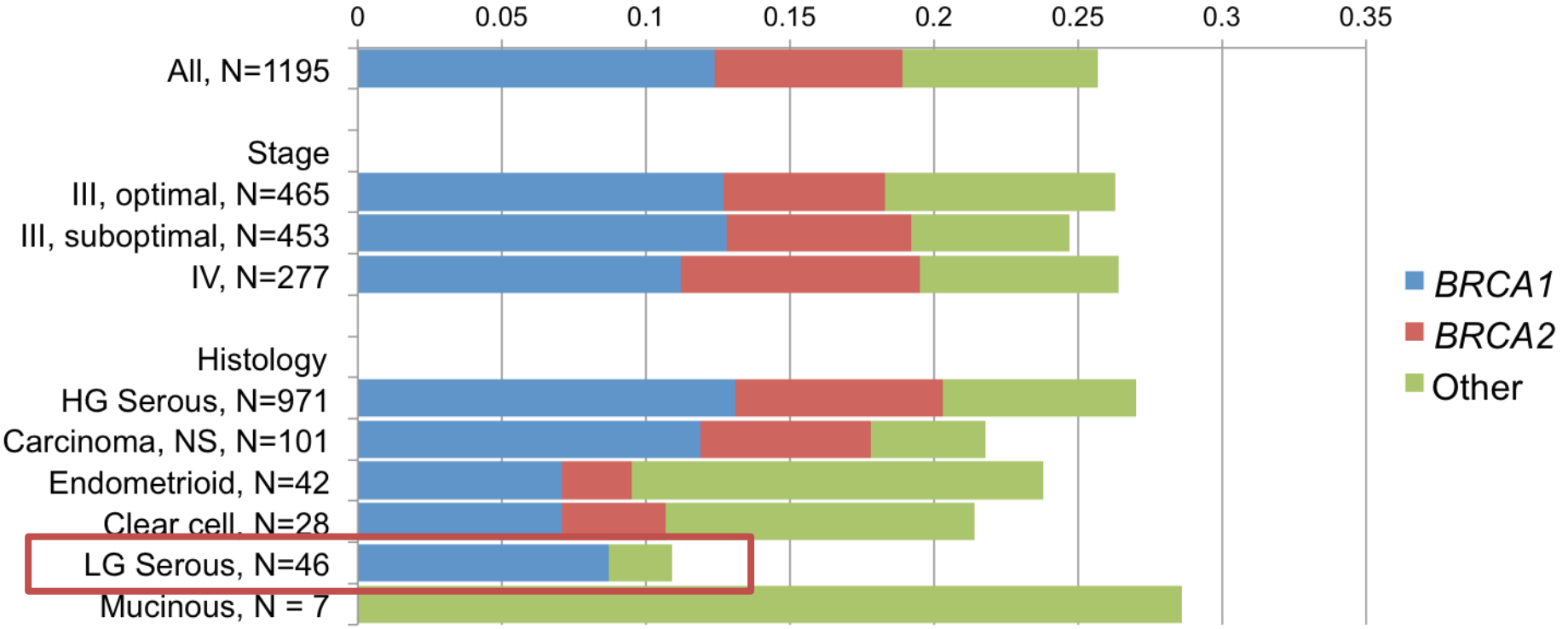


N = 1195

Clinical characteristics by mutation status

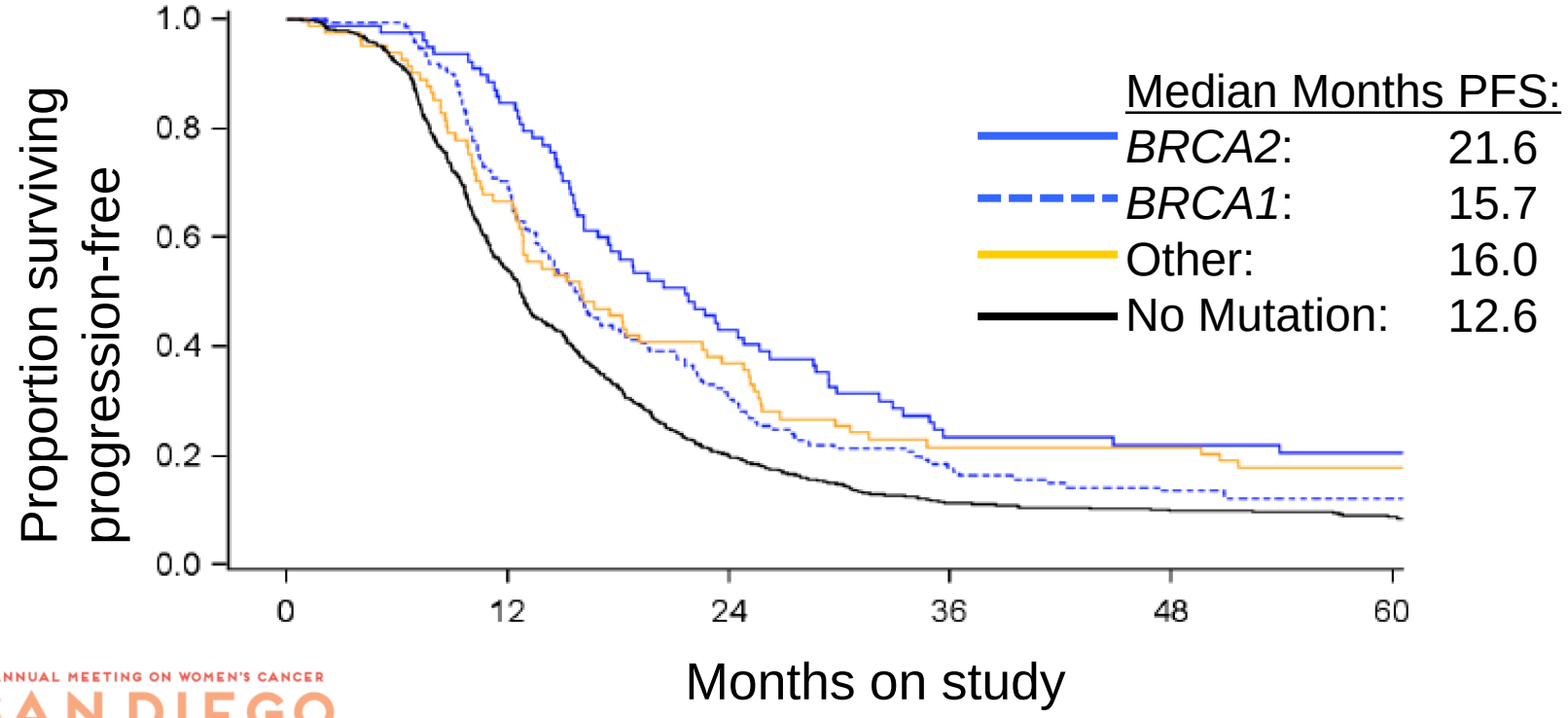


Clinical characteristics by mutation status



Low-grade serous histology had a significantly lower mutation rate,10.9% versus 27.0%, (p=0.02, OR 0.33, 95% CI: 0.1 – 0.8)

Progression-free survival by mutation status

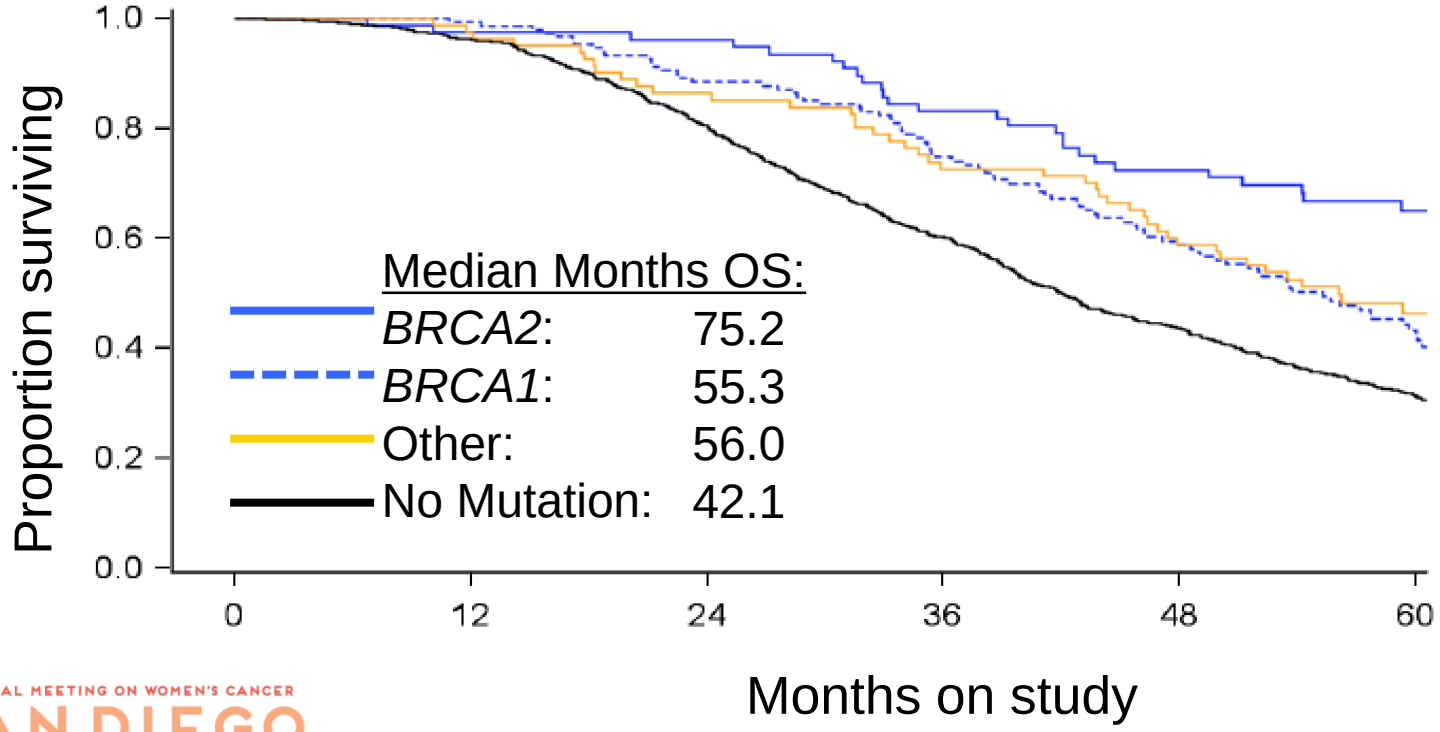


Estimated relative hazards for progression by mutation category

Mutation Category	Hazard Ratio (95% CI)	P-Value
BRCA2	0.52 (0.40 – 0.67)	<0.0001
BRCA1	0.80 (0.67 – 0.97)	0.02
Other HR	0.73 (0.57 – 0.94)	0.01

- Reference group is those with no mutation
- Hazard ratios are adjusted for study treatment, stage of disease, size of residual disease, initial performance status

Overall survival by mutation status

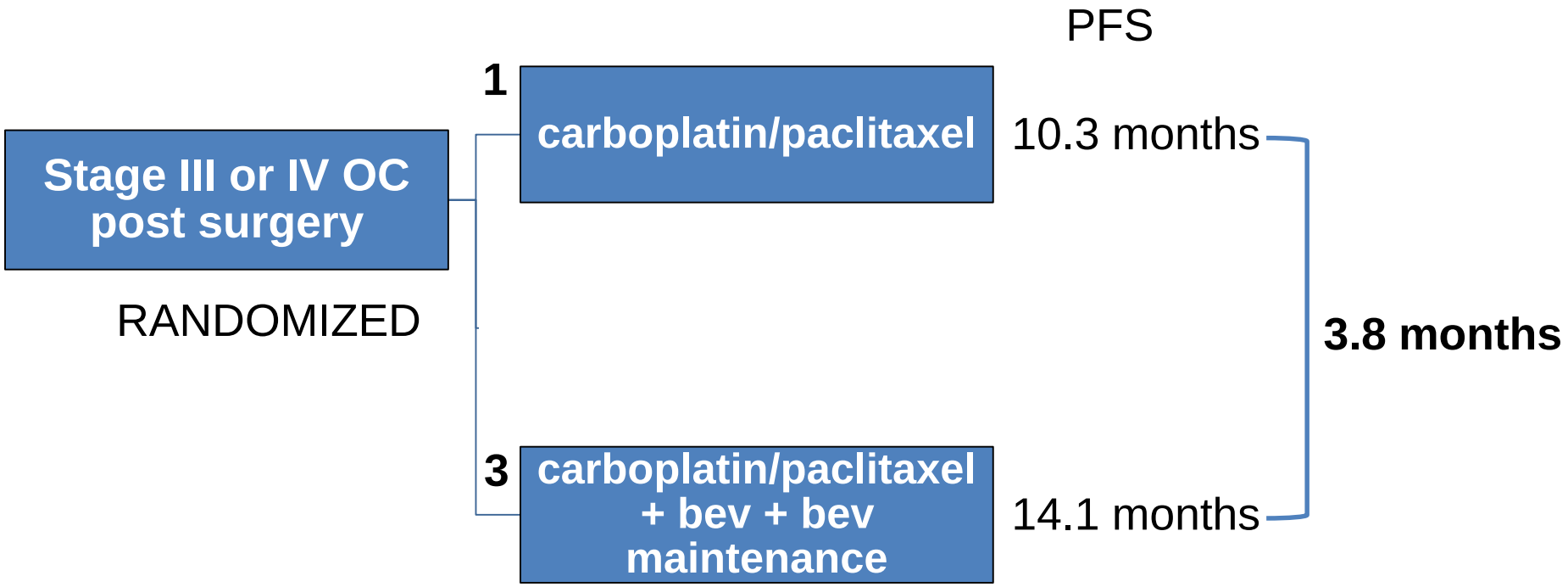


Estimated relative hazards of death by mutation category

Mutation Category	Hazard Ratio (95% CI)	P-Value
BRCA2	0.36 (0.25 – 0.53)	<0.0001
BRCA1	0.74 (0.59 – 0.94)	0.01
Other HR	0.67 (0.49 – 0.90)	0.007

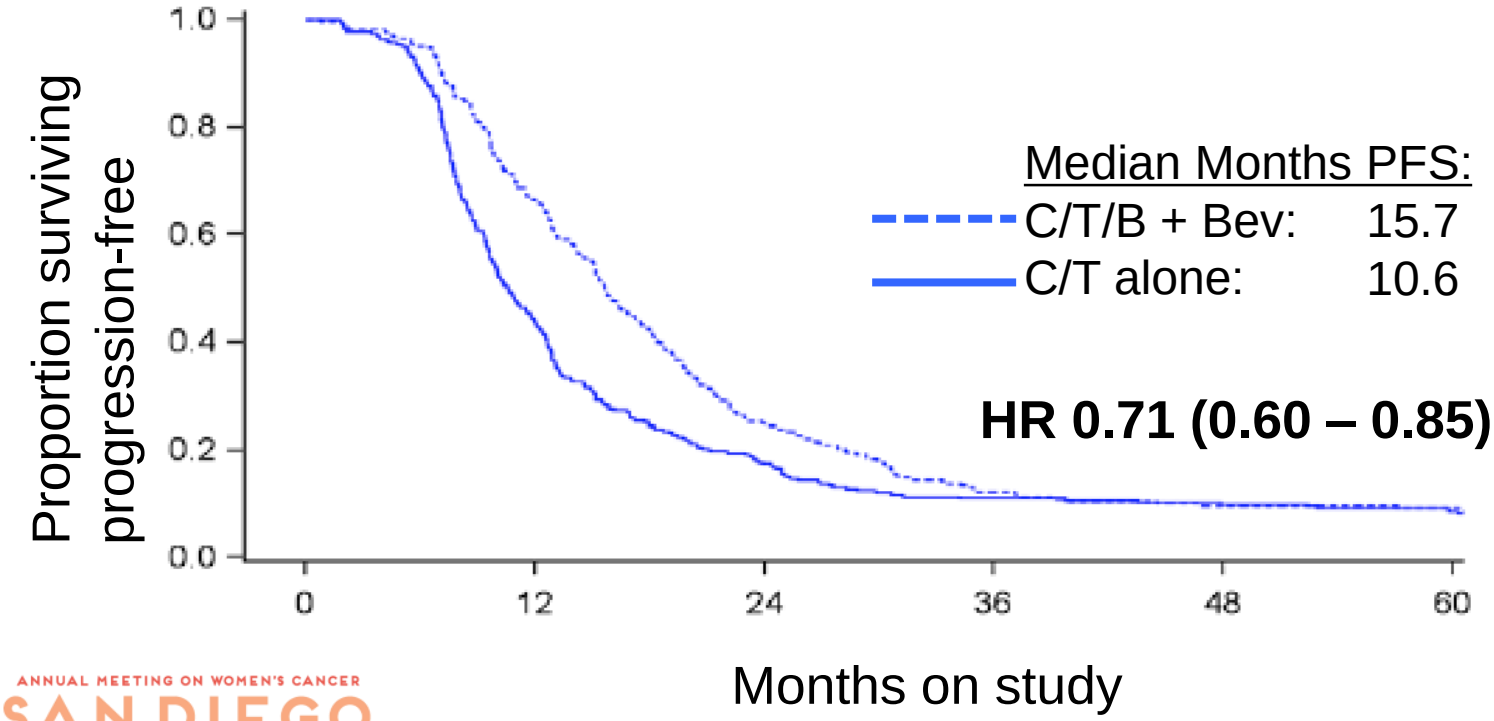
- Reference group is those with no mutation
- Hazard ratios are adjusted for study treatment, stage of disease, size of residual disease, initial performance status

Bevacizumab treatment effect by mutation category

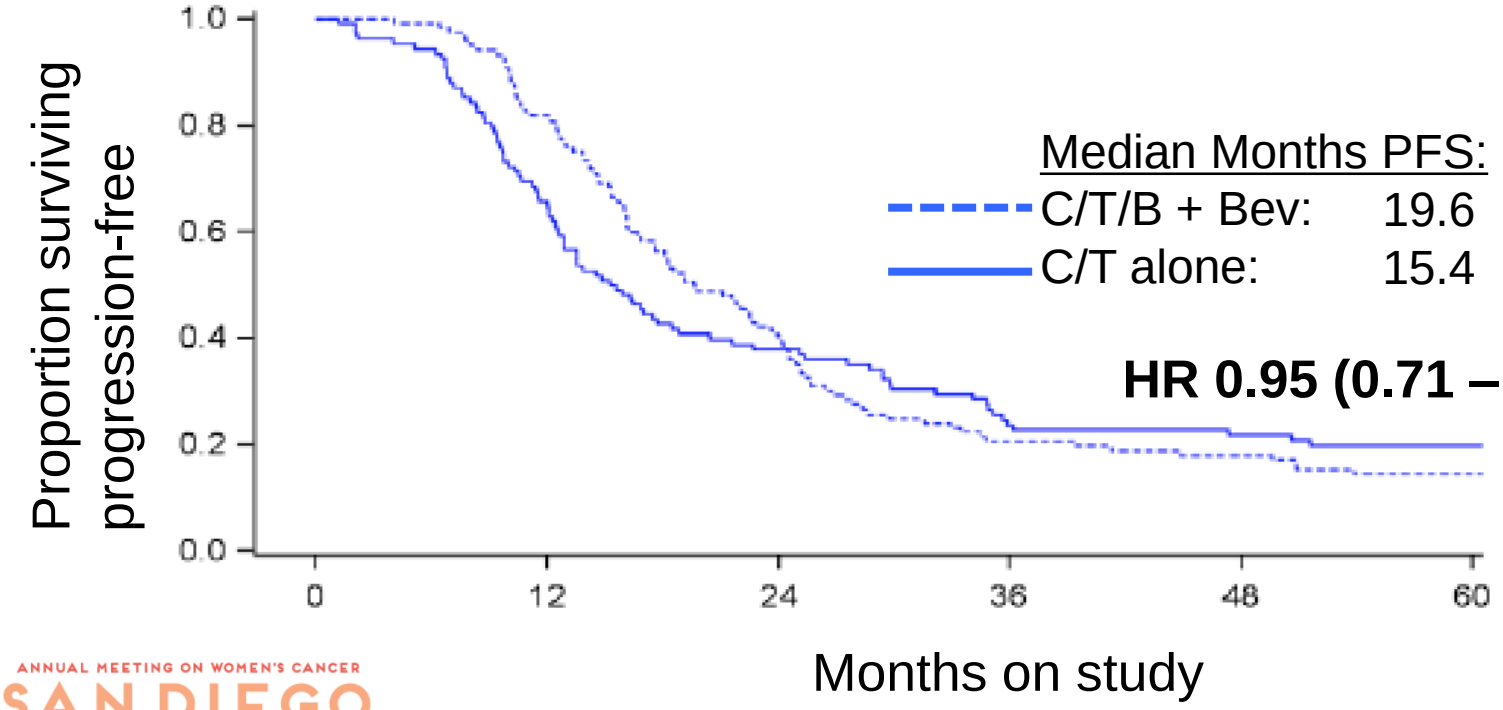


Arms 1 (C/T alone) and 3 (C/T/B + Bev): N = 809

Progression-free survival by treatment arm: no mutations (N = 581)



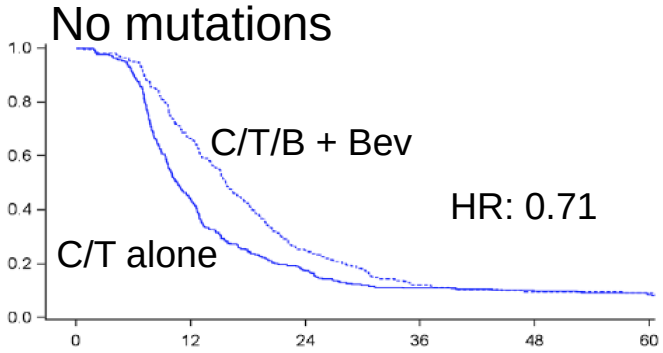
Progression-free survival by treatment arm: with mutations (N = 228)

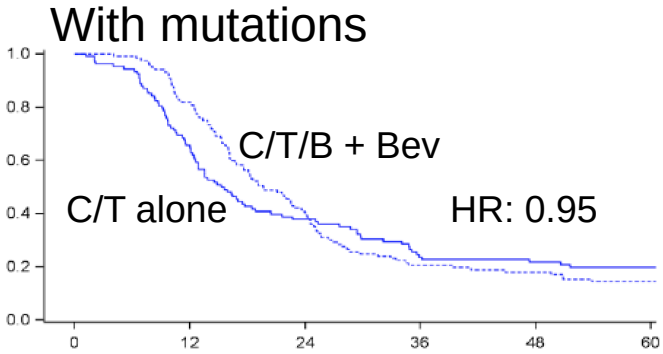


4.2 months

HR 0.95 (0.71 – 1.26), NS

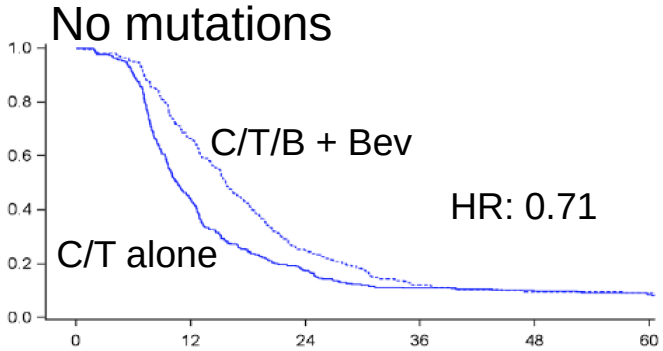
Did mutation status impact the effect of bevacizumab on progression?

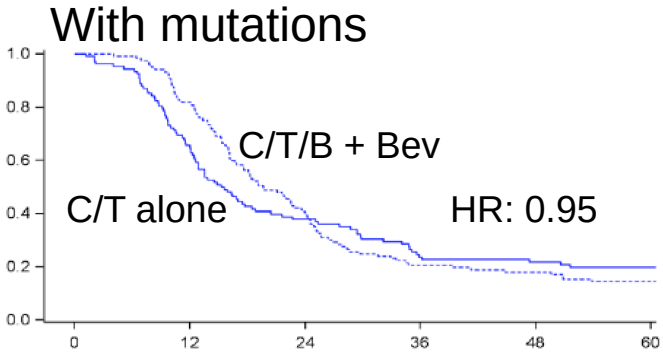




- Is mutation status an effect modifier?

Did mutation status impact the effect of bevacizumab on progression?





- Is mutation status an effect modifier?
- Test of interaction: (0.95/0.71) = 1.33, (0.95 – 1.85), p=0.098
- Mutation status did **not** significantly modify the effect of extended bevacizumab on PFS

Summary and Conclusions

- Women with OC with mutations affecting homologous recombination had significantly longer PFS and OS than those with no mutations
  - Important prognostic information for patients
  - Mutation status should be incorporated into clinical trial design

Summary and Conclusions

- Mutations affecting homologous recombination were found with all histologic subtypes of OC
  - All women with OC should be offered genetic testing
  - Clinical trials that focus on high-grade serous histology are missing a significant fraction of homologous recombination-deficient carcinomas
- Mutation status did not significantly modify the effect of bevacizumab on progression-free survival

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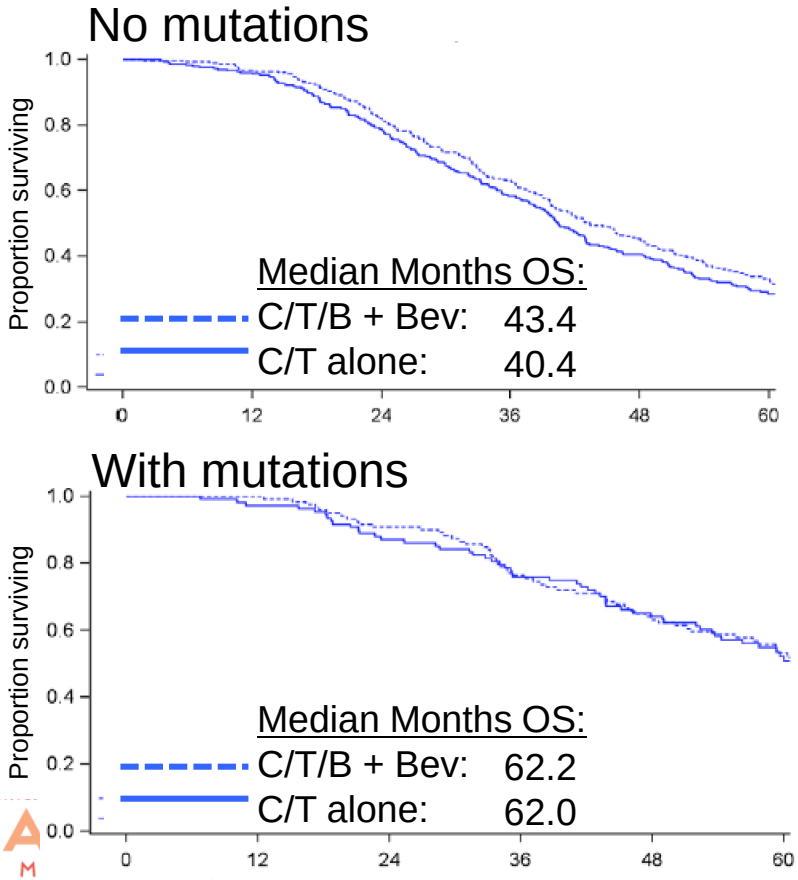
Overall survival by treatment arm



- No significant differences in overall survival with extended bevacizumab in those with or without mutations
- No evidence that mutation status is affecting the impact of bevacizumab on overall survival, test for interaction: p=0.53